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ORIGINAL PAPER

Wood anatomical changes due to uptake of cadmium and lead from contaminated soils in *Acer velutinum* seedlings

Zeynab Shahpoori • Vilma Bayramzadeh • Vahid Reza Safdari
Manoochehr Khan Zarinkafsh • Pedram Attarod • Roghayeh Jirroodnejad

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Abstract: We investigated wood anatomical changes due to uptake and accumulation of cadmium and lead from contaminated soils in *Acer velutinum* Boiss seedlings. Two-year old seedlings were exposed for 180 days to soil concentrations with varying cadmium and lead concentrations. We measured three wood anatomical traits, average vessel area (μm^2), vessel number per square millimetre, and vessel lumen area percentage (%). For assessing the cadmium and lead accumulation, we measured the concentrations in the soil, leaf, stem, and root. Average vessel area and vessel lumen area percentage were similar ($p>0.05$) in control and treated seedlings. Vessel number per square millimetre showed a decreasing trend from pith to bark in control and treated seedlings, and the trend was more pronounced in treated seedlings. We conclude that vessel number per square millimetre in *A. velutinum* is influenced by soil contamination. *A. velutinum* Boiss is not a suitable species for remediation of soils contaminated by cadmium and lead but it can be used as an indicator of the soil lead contamination, because lead concentrations in seedlings increased with increasing amount of lead in the soil.

Keywords: *Acer velutinum*; Cadmium; Lead; Phytoremediation; Vessel elements

Introduction

Heavy metal pollution is placing an ever-increasing load on the various resources of our environment, including soil. Soils contaminated by heavy metals can be found near industrial areas, metropolises, major transportation routes, roads, and areas treated with waste-water sludge. Cadmium and lead pollutants are considered among the most serious environmental problems worldwide (Folgar et al. 2009; Zacchini et al. 2009). Compared with other heavy metals, cadmium and lead are not essential nutrients for higher plants (Benavides et al. 2005; Zhao et al. 2005), and the exposure to relatively low concentrations, results in high toxicity to plants and animals (Benavides et al. 2005; Nedjimi and Daoud 2009). There is a need to develop efficient techniques for cadmium and lead removal from soil, water and air.

Fortunately, a variety of engineering and biology technologies have been developed to remediate contaminated ecosystems (Makino et al. 2006; McGrath et al. 2006). Among others, phytoremediation, using plants to remove pollutants from the environment or to render them harmless (Salt et al. 1998), is regarded as an alternative with great potential for affordable remediation of polluted sites (Prasad 2003; Sun et al. 2009). Some characteristics of plants, e.g. their ability to grow on nutrient-poor soils, deep rooting systems, rapid growth, metal-resistance, economically feasible secondary use, high biomass production, and sequestration of metals from the food chain (Pulford and Watson 2003; Punshon et al. 1996) are advantageous for phytoremediation. Trees meet all of these requirements, in particular the latter three characteristics (Pulford and Watson 2003). In addition, wood and bark are considered to be important sinks for biologically available metals, with additional sink tissue being formed each growing season. These tissues slowly incorporate into the decomposition cycle. Accumulated metals can be immobilized in a metabolically inactive compartment for a considerable period of time (Lepp 1996). Therefore, the potential for use of trees as vegetation cover on lands contaminated by heavy metals has received increasing attention over the last 15 years (Glimmerveen 1996; EPA 1999, 2000). These studies focused on cadmium and lead accumulation and growth responses in trees (Nedjimi and Daoud 2009; Shi and Cai 2009; Han et al. 2007). However, limited information is available on the potential for

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Zeynab Shahpoori • Vilma Bayramzadeh (✉) • Vahid Reza Safdari
Manoochehr Khan Zarinkafsh • Roghayeh Jirrodnejad
Department of Soil Sciences, Karaj Branch, Islamic Azad University, Karaj, Iran.
Email: v.bayramzadeh@kiau.ac.ir and v_bayramzadeh@yahoo.com

Pedram Attarod
Departments of Forestry and Forest Economics, Faculty of Natural Resources, University of Tehran, Karaj, Iran

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secondary use of trees utilized for remediation of cadmium and lead contamination.

Because of increasing demand for wood products coupled with continuous increases in soil pollution by cadmium and lead, the feasibility of secondary use of trees grown on remediation sites is becoming more important. Potential end-product uses of tree biomass include fuel, raw material for paper production, a source of viscose for the textile industry and veneer factories (McElroy and Dawson 1986). Trees grown on contaminated soils should have economically useful wood quality and biomass after harvesting. Accordingly, changes in wood properties due to uptake of contaminants, for example changes in basic density, fiber, and vessel element dimensions should be evaluated for trees grown on polluted soils. Lamoreaux and Chaney **(**1997) reported reduction in dimensions of vessels and tracheids, and partial blockage of xylem elements under cadmium stress in silver maple, and their findings are discussed in relation to water stress. Attention to changes in wood properties and the feasibility of secondary use, however, was ignored.

The objectives of our study were firstly to evaluate the ability of *A. velutinum* Boiss to remove cadmium and lead from contaminated soils, and secondly to document changes in different characteristics of the vessel elements in *A. velutinum* seedlings exposed to contaminated soils.

We experimented with *A. velutinum* because it is one of the most important tree species in the timber industry. Moreover, it is a fast growing species in coastal forests subjected to contamination by cadmium and lead due to the proximity of industrial centers.

Materials and methods

Plant materials and experimental design

Two-year old *A. velutinum* seedlings (about 47 cm in height and 7 mm in diameter at the base) were prepared for the pot growth experiment. Seedlings were transplanted in plastic pots (20 cm in height and 23 cm in diameter), one individual per pot. Each pot was filled with 4 kg soil. Soil characteristics are listed in Table 1. The seedlings were exposed to the following cadmium chloride and lead nitrate concentrations: 0 (control), 8, 16, 32, 64 mg· kg^{-1} of soil in early March, 2010, and plants were harvested after 180 days. The experiment was performed in the completely randomized design, and each treatment was replicated four times.

Table1. Soil characteristics

Parameter	pH (H_2o)	EC[1)] (ds/m)	Total Nitrogen (%)	Phosphorus (mg·kg^{-1})	Potassium (mg·kg^{-1})	$CaCO_3$ (%)	OM[2)] (%)	Pb[2)] (mg·kg^{-1})	Cd[2)] (mg·kg^{-1})
Silty-loam	7.43	1.251	0.266	319.8	1520	20	6.02	4.7	4.5

1) EC, electrical conductivity; 2) OM, Organic matter; Pb, lead; Cd, Cadmium

Soil samples were air-dried and ground to enable passage through a 2-mm sieve. Soil analyses were carried out using the following methods: particle size distribution by the hydrometer method (Gee and Bauder 1986); organic matter (OM) content by the Walkley-Black procedure (Nelson and Sommers 1982); and pH values by a glass electrode in mixture of soil and deionized water (1:5, w/v). For determining total cadmium and lead concentrations, soil samples were digested with a concentrated acid mixture of HNO_3-$HClO_4$ and heated at 95° C for 2 h. After cooling, the extract was diluted, filtered, and made up to 50 mL with 1% HNO3. Cadmium and lead concentrations of the extract were determined by an atomic absorption spectrometer.

Measuring anatomical characteristics of the vessel elements

Whole plants were separated into roots, stems, and leaves, washed with tap water and rinsed three times with deionized water. For anatomical studies, a 5-cm disc was cut from the main stem 5 cm above the base. Transverse sections of 14 μm in thickness were made using a sliding microtome. The sections were stained with safranin, dehydrated through a series of ethanol baths, and then mounted on slides with Canada balsam. The images were obtained by a digital camera (Sony DSC-W130) connected to a light microscope (Nikon). Wood anatomical characteristics, such as vessel number per square millimeter, average vessel area and percentage of vessel lumen area, in the annual rings from pith to bark side were measured by image analysis software, ImageJ (National Institutes of Health, Maryland, USA; Bayramzadeh et al. 2008). Cross-sectional areas of 1 mm^2, in the center of annual rings, excluding broad and narrow rays, were selected to measure the vessel element parameters. The threshold of cell area for defining vessel elements to distinguish them from wood fibers or axial parenchyma cells was a lumen area of 25 μm^2. This decision was made according to the frequency distribution of porous areas in all annual rings of one sample (data not shown). Since vessel elements were not exactly circular, the areas were calculated as ellipses.

For calculating the vessel number per square millimeter, vessel elements were counted in the mentioned areas, and then numbers per square millimeter were obtained for each annual ring (Bayramzadeh et al. 2008).

Calculations

Roots, stems, and leaves were rinsed in distilled water and then dried at 76° C for 48 h to constant weight. Dried plant material was ground in a stainless steel mill to fine powder. The powders were digested with $HClO_4$ and heated at 80° C. After cooling, the extract was diluted, filtered, and made up to 50 mL with distilled water. Concentrations of cadmium and lead in roots, stems, and

leaves were determined using an atomic absorption spectrometer.

The bioaccumulation coefficient (BC) or enrichment factor was calculated according to Liu et al. (2009) and Tanhan et al. (2007) as the ratio of cadmium or lead concentration in the whole plant to the cadmium or lead concentration in the soil. The translocation factor (TF) indicates the ability of plants to translocate cadmium and lead from the roots to the shoots (Mattina et al. 2003). TF for cadmium and lead concentration was calculated according to Liu et al. (2009) as a ratio of cadmium or lead concentration in shoots to the cadmium or lead concentration in roots. The tolerance index (Ti) was calculated to measure the ability of the plant to grow in the presence of a given concentration of metal (Zacchini et al. 2009; Wilkins 1978). Ti is the ratio of dry weight of plants grown in soils contaminated by cadmium or lead to the dry weight of plants grown in uncontaminated control soils.

Statistical analysis

All statistical analyses were performed using the StatGraphics plus 5.1 (stat point, Inc., Northern Virginia, USA). One-way ANOVA was carried out to assess the difference of means by Tukey's Honestly Significant Difference (HSD).

Results and Discussion

Growth responses and wood anatomical changes

Neither height nor basal diameter of *A. velutinum* seedlings differed significantly ($p < 0.05$) between control and treated groups prior to exposure to lead and cadmium soil pollution (Table 2).

Basal diameter differed noticeably between control and treated seedlings after exposure to cadmium. However the rate of increase in basal diameter was not statistically different ($p < 0.05$) between control and treated plants.

Height of control versus treated *A. velutinum* seedlings did not differ ($p < 0.05$) after exposure to lead or cadmium (Table 2).

Dry weights of stems, leaves, and roots were similar for control and treated seedlings, except that dry weight of roots in lead treated soil (Table 3) was significantly different after exposure. However, a decline in dry weight of roots was not apparent with increasing lead concentration in the soil (Table 3).

Table 2. Height (cm) and base diameter (mm) of *Acer velutinum* after and before exposing to lead and cadmium soil pollutions

Cadmium concentration	Height (cm)		Base diameter (cm)		
($mg \cdot kg^{-1}$)	Before exposing	After exposing	Before exposing	After exposing	Increasing rate
0	43.5±(10.25)^{a}	46.72±(9.6)^{a}	6.33±(1)^{a}	6.8±(0.91)^{a}	0.47±(0.08)^{a}
8	47.16±(16.4)^{a}	51.36±(18.99)^{a}	8.75±(4.53)^{a}	8.83±(3.21)^{b}	0.08±(0.01)^{a}
16	50.09±(13.34)^{a}	51.47±(17.31)^{a}	8.22±(2.1)^{a}	9.2±(1.47)^{ab}	0.98±(0.62)^{a}
32	53±(9.74)^{a}	54.17±(9.38)^{a}	8.55±(2)^{a}	8.6±(0.94)^{ab}	0.05±(0.02)^{a}
64	43.33±(18.19)^{a}	47.39±(18.39)^{a}	7±(2.83)^{a}	7.33±(2.13)^{ab}	0.33±(0.02)^{a}
Lead concentration	Height (cm)		Base diameter (cm)		
($mg \cdot kg^{-1}$)	Before exposing	After exposing	Before exposing	After exposing	
0	43.5±(10.25)^{a}	46.72±(9.6)^{a}	6.33±(1)^{a}	6.8±(0.91)^{a}	
8	50.5±(11.48)^{a}	54.17±(12.75)^{a}	8.66±(2.74)^{a}	9±(1.85)^{a}	
16	45.72±(11.61)^{a}	49.21±(12.48)^{a}	7.91±(2.15)^{a}	7.91±(2.19)^{a}	
32	49±(10.85)^{a}	51.47±(11.15)^{a}	8.45±(2.58)^{a}	8.65±(1.95)^{a}	
64	41.8±(13.28)^{a}	42.47±(12.56)^{a}	6.2±(0.57)^{a}	6.6±(0.54)^{a}	

Means with the different letters (a&b) are significantly different at $p<0.05$ by Tukey's HSD procedure; Mean± SD

Therefore, it seems that growth of *A. velutinum* seedling was not affected by the experimental levels of cadmium and lead contamination. In contrast to these results, Ahmad et al. (2005) reported reduction in whole plant length, shoot length, and dry weight of shoot in *Trigonella foenum graecum* Linn. Therefore, it seems that these varied results are likely to be associated with species-specific differences.

Data for anatomical characteristics of vessel elements are shown in Figs. 1 to 3. Average vessel lumen area (Fig. 1A and 1B) increased with seedling age in both control and treated seedlings (from annual rings number 1 to 3) as expected. This characteristic was not different ($p<0.05$) in control versus treated seedlings in the studied annual rings from pith side to bark side, separately. Therefore, average vessel lumen area was not significantly affected by soil cadmium or lead contamination. This finding for *A. velutinum* differed from findings of Lamoreaux and Chaney (1997) and Khudsar et al. (2000) for silver maple (*Acer saccharinum*) and *Cajanus cajan*. The conflicting results may be attributed to the spices-specific differences or dissimilarity of media (soil and water). However, this result confirms the results of Bayramzadeh et al. (2008), who reported that average vessel element lumen area is not sensitive to environmental conditions in diffuse porous hardwoods such as *A. velutinum*.

Table 3. *Acer velutinum* biomass and its components after exposing of different cadmium and lead supplies

Cadmium supply ($mg \cdot kg^{-1}$)	Leaf biomass (g)	Shoot biomass (g)	Root biomass (g)	Lead supply ($mg \cdot kg^{-1}$)	Leaf biomass (g)	Shoot biomass (g)	Root biomass (g)
0	$0.43 \pm (0.18)^a$	$7.97 \pm (3.67)^a$	$8.86 \pm (1.62)^a$	0	$0.43 \pm (0.18)^a$	$7.97 \pm (3.67)^a$	$8.86 \pm (1.62)^a$
8	$0.62 \pm (0.31)^a$	$9.25 \pm (2.39)^a$	$13.51 \pm (5.90)^a$	8	$0.58 \pm (0.19)^a$	$7.58 \pm (3.45)^a$	$18.05 \pm (2.66)^b$
16	$0.40 \pm (0.24)^a$	$4.72 \pm (1.16)^a$	$6.66 \pm (1.82)^a$	16	$0.48 \pm (0.18)^a$	$7.46 \pm (1.15)^a$	$9.83 \pm (2.02)^c$
32	$0.44 \pm (0.16)^a$	$7.74 \pm (2.38)^a$	$9.02 \pm (3.09)^a$	32	$0.50 \pm (0.18)^a$	$9.05 \pm (3.22)^a$	$11.37 \pm (4.47)^d$
64	$0.42 \pm (0.22)^a$	$5.3 \pm (1.03)^a$	$8.72 \pm (2.10)^a$	64	$0.33 \pm (0.10)^a$	$6.44 \pm (3.55)^a$	$6.91 \pm (2.79)^e$

Means with the different letters (a, b, c, d and e) are significantly different at $p<0.05$ by Tukey's HSD procedure; Mean± SD

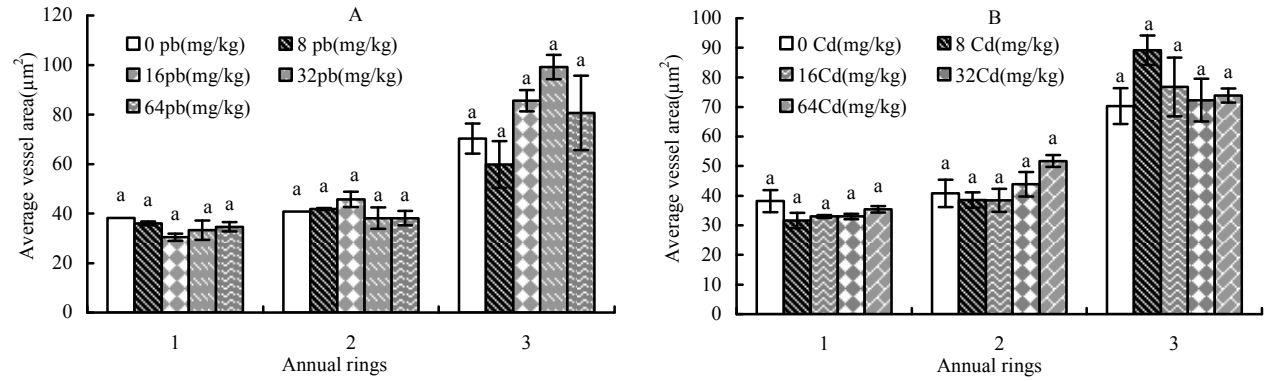

Fig. 1 Average vessel elements lumen area from pith (annual ring number one) to bark (annual ring number three) in control and treated seedlings by lead (A) and cadmium (B); Means with the same letter are not significantly different at $p<0.05$ by Tukey's HSD procedure.

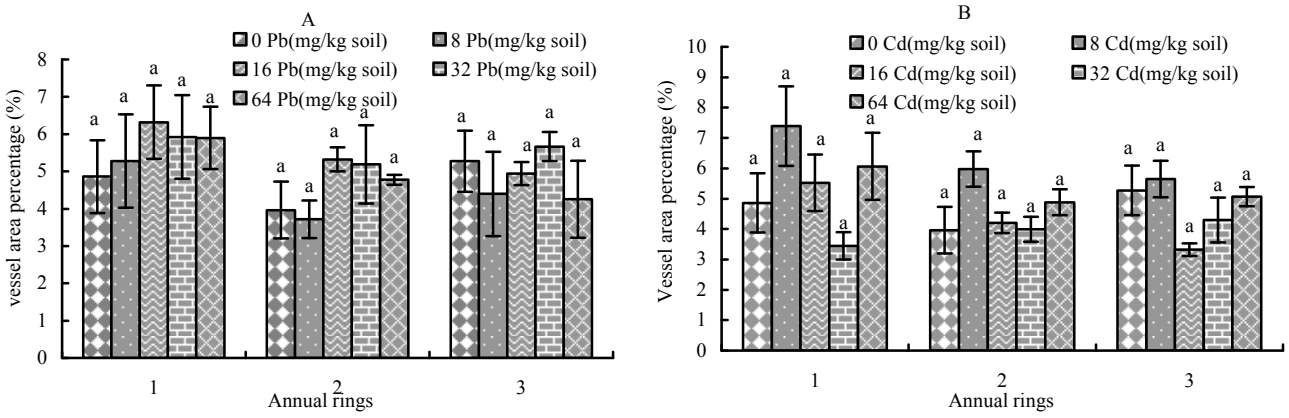

Fig. 2 Vessel lumen area percentage from pith (annual ring number one) to bark (annual ring number three) in control and treated seedlings by lead (A) and cadmium (B); Means with the same letters are not significantly different at $p<0.05$ by Tukey's HSD procedure.

Vessel area percentage was similar ($p<0.05$) between control and treated seedlings in all annual rings from the pith side to the bark side (Fig. 2A and 2B). Vessel lumen area percentage was not affected by soil cadmium or lead contamination.

Vessel number per square millimetre (Fig. 3A and 3B) showed a decreasing trend from pith to bark in control and treated seedlings as expected. However, it was more significant in treated seedlings. Maximum and minimum decreasing rates in vessel number per square millimetre were detected for 8 mg cadmium (113%), 32 mg lead (97%) and control (32%). Vessel number per square millimetre was affected by soil cadmium and lead contamination. This is in conformity with the observations of Ghouse and Yunus (1972) and Khudsar et al. (2000), who reported that air and soil pollution can cause a decrease in vessel abundance in several angiosperm herbs and shrubs. In diffuse porous hardwoods, vessel number per square millimetre is sensitive to growth conditions. This finding can be related to the fact that the pollutants affect cell division directly or indirectly, which seems to be essential for both the production of procambium , cambium and the differentiation of cambium cells into vessels and fibers (Aloni 1987).

Cadmium and lead accumulation

Cadmium concentration in root and leaf tissues increased with increasing cadmium concentration in soils, but cadmium concentration in shoots showed no significant difference (p> 0.05). Lead concentration in root, shoot, and leaf increased noticeably with the increase of lead supply in soil (Table 4).

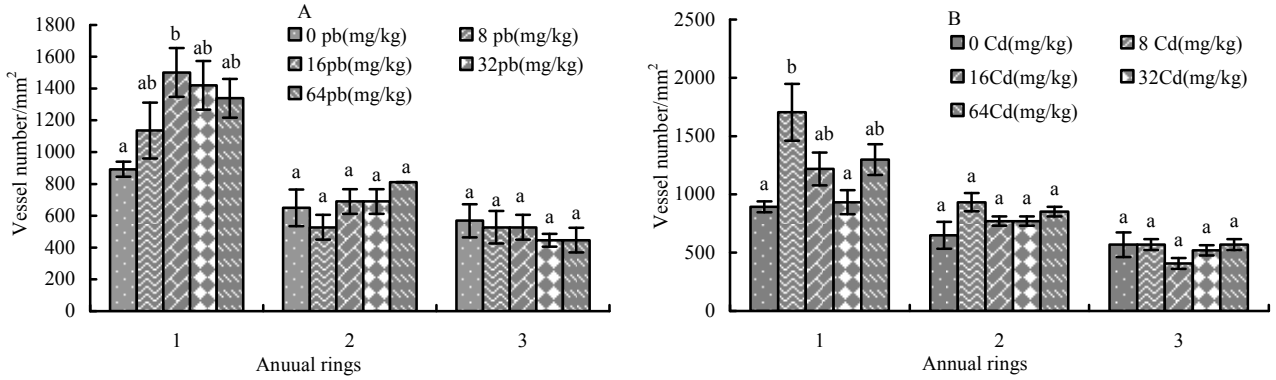

Fig. 3 Vessel number per square millimeter from pith (annual ring number one) to bark (annual ring number three) in control and treated seedlings by lead (A) and cadmium (B); Means with the different letters(a&b) are significantly different at P<0.05 by Tukey's HSD procedure.

Table 4. Cadmium and lead concentration in *Acer velutinum* and its components

Cadmium supply (mg×kg^{-1} soil)	Cd concentration in the leaf (mg· kg^{-1} plant)	Cd concentration in the shoot (mg· kg^{-1} plant)	Cd concentration in the root (mg· kg^{-1} plant)	Total (mg· kg^{-1} plant)
0	0.19 ± (0.007)^{a}	1.2 ±(0.35)^{a}	0.21 ±(0.04)^{a}	1.6 ±(0.46)^{a}
8	0.19 ± (0.006)^{a}	1 ±(0.49)^{a}	0.91 ±(0.01)^{a}	2.1 ±(1.02)^{a}
16	0.26 ± (0.02)^{b}	1.5 ±(0.45)^{a}	1.8 ± (0.33)^{ab}	3.56 ± (0.64)^{ab}
32	0.42 ± (0.01)^{c}	0.85 ± (0.33)^{a}	2.5 ± (1.55)^{b}	3.77 ± (2.2)^{b}
64	0.99 ± (0.06)^{d}	1.3 ± (0.52)^{a}	5.9 ± (1.12)^{c}	8.19 ± (1.5)^{c}
Lead supply (mg×kg soil)	Pb concentration in the leaf (mg· kg^{-1} plant)	Pb concentration in the shoot (mg· kg^{-1} plant)	Pb concentration in the root (mg· kg^{-1} plant)	Total (mg· kg^{-1} plant)
0	0.19 ±(0.007)^{a}	0.62 ±(0.75)^{a}	1.04 ±(0.53)^{a}	1.85 ±(0.91)^{a}
8	1.45 ±(0.004)^{a}	1 ±(1.68)^{b}	8.87 ±(3.88)^{a}	11.32 ±(4.8)^{a}
16	2.74 ±(0.05)^{b}	3.5 ±(2.16)^{b}	17.37 ±(4.53)^{a}	23.61 ±(8.8)^{a}
32	5.64 ±(0.05)^{c}	5.27 ±(3.37)^{a}	34.37 ±(10.66)^{ab}	45.28 ±(10.9)^{ab}
64	11.32 ±(0.04)^{d}	8.12 ±(6.5)^{a}	66.17 ±(16.02)^{b}	85.61 ±(13.6)^{b}

Means with the different letters (a, b, c, d and e) are significantly different at p<0.05 by Tukey's HSD procedure; Mean± SD

Previous studies reported that plants experience toxic effects when cadmium and lead concentrations in tissues reaches 3–10 mg· kg^{-1} (Shi and Cai 2009; Balsberg-Pahlsson 1989) and 30–300 mg· kg^{-1} (Alloway 1990) dry weight, respectively. Our results suggest that *A. velutinum* experienced toxic effects from cadmium and lead contaminated soils because cadmium and lead in plant tissues was 8.19 mg· kg^{-1} and 85.61 mg· kg^{-1} dry weight, respectively (Table 4). Nevertheless, *A. velutinum* showed only slight declines in growth and biomass production as cadmium and lead contamination level increased (Table 3).

Adaptive responses in morphology and biomass production are the primary tolerance indicators, by which *A. velutinum* can cope with the cadmium and lead soil contamination. Earlier studies suggested that four indicators could be used to define a hyperaccumulator: (1) the threshold value (>100 mg· kg^{-1} dry weight for cadmium and > 1,000 mg· kg^{-1} dry weight for lead) (Baker 1981; Zhou and Song 2004; Baker and Brooks 1989; Chaney et al. 1997); (2) BC; (3) TF; and (4) Ti (Sun et al. 2009; Zhou and Song 2004). This threshold value of cadmium was not appropriate in our study (Table 5). BC did not increase with the increased concentration of cadmium in soil and it was less than one (Table 5). In cadmium treatments, TF decreased with increased cadmium concentrations in soil. The results of TF indicated that *A. velutinum* could not transport cadmium from the root to the shoot at high concentrations, because plants have strategies for overcoming environmental risks of cadmium (Raskin et al. 1994; Hart et al. 2002). The results from Ti point to the conclusion that Ti was not significantly reduced with increasing cadmium concentrations in soil. Even so, the synthesized analysis implied that *A. velutinum* could not be a cadmium hyperaccumulator in the soil with regard to threshold values, Ti < 1, TF < 1.0 (excluding control and 8 mg· kg^{-1}), and BC < 1.0.

BC, TF and Ti in lead contaminated soil showed the following order: BC< 1 (except in 64 mg lead), TF<1, Ti≥1 (except in 64

mg lead) and the threshold value<1,000 mg· kg^{-1} dry weight for lead. The results of TF revealed that *A. velutinum* could not transport more lead from root to shoot in the contaminated soil. BC in 64 Pb mg· kg^{-1} soil· was more than one (Table 5). Also, BC increased with increasing lead concentration in the soil (Tables 4 and 5). *A. velutinum* showed more capacity to accumulate lead with increasing lead concentration in the soil. According to the result, *A. velutinum* could not be a lead hyperaccumulator in the studied soil. However, it might be used as indicator of lead, since the concentration of lead in plant tissues increased with increasing lead concentrations in the soil (Ghosh 2005).

Therefore, among the wood anatomical characteristics vessel number per square millimeter was very sensitive to cadmium and lead contaminations, similar to other diffuse porous hardwoods. *A. velutinum* could not be cadmium or lead hyperaccumulator in the studied soil. However, it might be used as indicator of lead.

Table 5. Bioaccumulation coefficient (BC), transport factor (TF) and tolerance index (Ti) of *Acer velutinum* , (means ± SD) in respond to different Cadmium(Cd) and Lead(Pb) supplies

Concentration	Bioaccumulation coefficient(BC)		Transport factor (TF)		Tolerance index (Ti)	
(mg· kg^{-1})	Cd	Pb	Cd	Pb	Cd	Pb
Control (0)	0.35 ±(0.17)^a	0.35 ±(0.17)^a	5.95 ±(1.6)^a	0.6 ±(0.72)^a	1 ±(0.26)^a	1 ±(0.26)^a
8	0.22 ± (0.14)^a	0.26 ±(0.3)^a	1.09 ±(0.87)^a	0.11 ±(0.17)^b	1.35 ±(0.36)^a	1.51 ±(0.49)^a
16	0.27 ± (0.18)^a	0.48 ±(0.5)^a	0.81 ±(0.23)^a	0.2 ±(0.12)^b	0.68 ±(0.17)^a	1.03 ±(0.27)^a
32	0.17 ±(0.15)^a	0.83 ±(0.91)^a	0.33 ±(0.12)^a	0.15 ±(0.09)^a	0.99 ±(0.35)^a	1.21 ±(0.33)^a
64	0.23 ±(0.21)^a	1.35 ±(1.5)^a	0.23 ±(0.08)^a	0.12 ±(0.09)^a	0.83 ±(0.24)^a	0.79 ±(0.2)^a

Means with the different letters (a and b) are significantly different at $p < 0.05$ by Tukey's HSD procedure.

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